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Semimechanistic Pharmacokinetic-Pharmacodynamic Model with Adaptation Development for Time-Kill Experiments of Ciprofloxacin against *Pseudomonas aeruginosa*▽

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The objective of this study was to implement a semimechanistic pharmacokinetic-pharmacodynamic (PK-PD) model to describe the effects of ciprofloxacin against *Pseudomonas aeruginosa* in vitro. Time-kill curves were generated with an initial inoculum close to 5×10^6 CFU/ml of *P. aeruginosa* PAO1 and constant ciprofloxacin concentrations between 0.12 and 4.0 μg/ml (corresponding to 0.5× and 16× MIC). To support the model, phenotypic experiments were conducted with the PAO7H mutant strain, which overexpresses the MexEF OprN efflux pump and phenyl arginine β-naphthylamide (PAβN), a known efflux inhibitor of main Mex multidrug efflux systems. A population approach was used for parameter estimation. At subinhibitory ciprofloxacin concentrations (0.12 and 0.25 μg/ml), an initial CFU decay followed by regrowth was observed, attesting to rapid emergence of bacteria with increased but moderate resistance (8-fold increase of MIC). This phenomenon was mainly due to an overexpression of the Mex protein efflux pumps, as shown by a 16-fold diminution of the MIC in the presence of PAβN in these strains with low-level resistance. A PK-PD model with adaptation development was successfully used to describe these data. However, additional experiments are required to validate the robustness of this model after longer exposure periods and multiple dosing regimens, as well as in vivo.

Pseudomonas aeruginosa is one of the leading pathogens involved in nosocomial pneumonia. Infections with *P. aeruginosa* are associated with significant morbidity and mortality. The increasing frequency of multidrug-resistant *P. aeruginosa* strains is a concern since effective antimicrobial options are severely limited (27). Fluoroquinolones (FQs) such as ciprofloxacin, which target the bacterial enzymes DNA gyrase and topoisomerase IV, which are essential for maintenance of the appropriate DNA topological state for replication and transcription may represent an effective therapy against *P. aeruginosa* (8). However, *P. aeruginosa* also becomes resistant to FQs through two well-documented and separate mechanisms: (i) expression of drug efflux pumps that reduce the accumulation of antibiotics in the cell and (ii) point mutations in the genes of the quinolone target enzymes, topoisomerase and gyrase (9, 12, 18, 29, 38).

Because amplification of a resistant subpopulation is dependent on the antibiotic dosing regimen (14), optimization of dosing regimens should allow the limiting of these phenomena. Time-kill studies are commonly used to address these issues by exploring the changes of bacteria counts in the presence of different concentrations of antibiotics in vitro, and pharmacokinetic-pharmacodynamic (PK-PD) indexes derived from the MIC are used to quantify the activity of antibiotics against bacteria (22). However, although very popular, these approaches present several limits when attempting to select the best dosing regimens, especially when bacterial regrowth is observed after an initial decay, as occasionally observed (7, 24, 25). A more complex but also more powerful way to assess the efficacy of antimicrobial agents is to use PK-PD modeling approaches and to characterize drug effects over the whole period of time (23).

Analysis of time-kill curves using PK-PD models could allow better characterization of the dynamics of the drug effect with time and concentration compared to simply using the MIC, as demonstrated on several occasions (7, 24–26). However such in vitro PK-PD experiments have generally been performed using devices allowing exposure of bacteria to variable antibiotic concentrations with time. Time-kill curve experiments that are performed at constant antibiotic concentrations and which are therefore easier to perform than experiments with concentrations that vary with time, have also been published recently (6, 25, 31, 34). In these experiments, data were analyzed by using nonlinear mixed-effect models, also called a "population approach." This methodology allows estimation of the mean PK-PD parameters as well as the interindividual and residual variability from a simultaneous modeling of the data from all individual experiments.

The objective of the present study was to select a PK-PD model to fit in vitro time-kill curves for *P. aeruginosa* exposed to different ciprofloxacin concentrations, using a population approach to estimate model parameters.

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MATERIALS AND METHODS

***P. aeruginosa* isolate.** A reference strain of *P. aeruginosa* PAO1, kindly provided by Patrice Nordman (INSERM U914, Université Paris-Sud, Hôpital de Bicêtre, Le Kremlin-Bicêtre, France), was used throughout the study. Strain PAO7H was generously provided by Didier Hocquet and Patrick Plesiat (Centre National de Référence de la Résistance aux Antibiotiques, Pseudomonas aeruginosa, Hôpital Jean Minjoz, Besançon, France). Bacteria were kept on plates containing Mueller-Hinton (MH) agar (Fluka Biochemika, Sigma-Aldrich, Lyon, France) between experiments.

Antimicrobial agents. A 200-mg/ml intravenous commercial solution of ciprofloxacin (ciprofloxacin IV; Panpharma, Fougères, France) was used throughout the study. A stock solution was prepared prior to each experiment by appropriate dilution of the antibiotic in sterile water at a concentration of 40 μg/ml.

Time-kill curve experiments. The *P. aeruginosa* inoculum was prepared by suspension of the bacteria from an 18-h logarithmic-growth-phase culture in Muller-Hinton (MH) broth (Fluka Biochemika, Sigma-Aldrich, France), adjusted to a final concentration of 5×10^6CFU/ml. The experiments were performed in 10-ml glass tubes with 4.5 ml of inoculum. Ciprofloxacin was added to obtain concentrations of 0.125, 0.25, 0.50, 1.0, 2.0, and 4.0 μg/ml (corresponding to 0.5× to 16× MIC). The tubes were incubated at 37°C for 24 h. Bacteria were counted at 0, 0.5, 1, 1.5, 2, 4, 6, 9, 18, 21, and 24 h. The number of CFU was counted after incubation at 37°C for 18 to 24 h. The limit of quantification (LOQ) was 100 CFU/ml. At least one growth control, performed without addition of ciprofloxacin, was included in each experiment. Overall, four different kill curves were performed for the control and 0.125- and 0.25-μg/ml concentrations and three curves were performed for concentrations of 0.5, 1, 2, and 4 μg/ml.

Analysis of bacterial populations. To support the PK-PD model, bacterial populations at 24 h after starting the time-kill curves experiments for control and ciprofloxacin concentrations equal to 0.125 and to 0.25 μg/ml were analyzed. Each suspension was directly plated on MH agar (Fluka BioChemika, Sigma-Aldrich, France) without ciprofloxacin or with increasing concentrations ranging from 0.125 μg/ml (0.5-fold MIC) to 4 μg/ml (16-fold MIC) (5). After 48 h of incubation at 37°C, the bacteria were counted and the percentage of surviving bacteria was determined. For each ciprofloxacin concentration tested, 10 single bacterial colonies were randomly selected and tested for ciprofloxacin susceptibility.

Ciprofloxacin susceptibility testing and detection of Mex protein active efflux. Ciprofloxacin MIC determination was performed in triplicate by the microdilution method in MH broth according the CLSI recommendations (http://www.clsi.org). To detect bacteria showing a reduced ciprofloxacin susceptibility due to an increased active efflux, the CIP MIC was measured in the absence and in the presence of 40 μg/ml of phenyl arginine β-naphthylamide (PAβN) (Sigma Aldrich, Milan, Italy), a known efflux inhibitor of main Mex multidrug efflux systems implicated in fluoroquinolone active efflux in *P. aeruginosa* (21, 28). A PAO7H mutant that overexpresses the MexEF OprN efflux pump was used as a positive control.

Analysis of the target modification mechanism. Two genes encoding the preferential targets of FQs in *P. aeruginosa*, *gyrA* encoding the A subunit of DNA gyrase and *parC* encoding the C subunit of topoisomerase IV, were amplified by PCR in their quinolone resistance determining region (QRDR) using previously described primers (2, 11). PCRs were carried out with 2 μl of an overnight broth culture, 0.2 μM each primer, and the Crimson *Taq* DNA polymerase (New England BioLabs). The thermal profiles were 95°C for 3 min, followed by 35 cycles of 30 s at 95°C, 20 s at 55°C, and 30 s at 68°C with a final extension of 5 min 68°C for *gyrA* PCR and 95°C for 3 min, followed by 35 cycles of 30 s at 95°C, 20 s at 61°C, and 30 s at 68°C with a final extension of 5 min 68°C for *parC* PCR. PCR products were purified using the Wizard SV gel and PCR cleanup system (Promega). Amplicons were sequenced with PCR primers using the Big Dye Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA).

PK-PD modeling. The experimental time-kill curves of ciprofloxacin against *P. aeruginosa* PAO1 were analyzed using a population approach. Data were log transformed before analysis, and when bacterial counts were below the limit of quantification (100 CFU/ml), a value of 50 CFU/ml was considered.

Structural model. An initial exponential bacterial growth followed by a plateau was observed in the absence of antibiotic and modeled according to equation 1 as previously described (24):

$$\frac{dN}{dt} = k_0\left(1 - \frac{N}{N_{max}}\right)N \quad (1)$$

where N is the number of viable bacteria, k_0 is the apparent growth rate constant, and N_{max} is the maximum number of bacteria obtained in the in vitro culture.

As ciprofloxacin exhibits a bactericidal activity (35, 37), a death stimulation model (37) corresponding to equation 2 was chosen to describe its effect:

$$\frac{dN}{dt} = k_0\left(1 - \frac{N}{N_{max}}\right)N - \frac{k_{max}C^\gamma}{EC_{50} + C^\gamma}N \quad (2)$$

where C is the ciprofloxacin concentration, k_{max} is the maximal rate of death, EC_{50} is the ciprofloxacin concentration that produces 50% of the maximum rate of death, and γ is the Hill's constant.

Given a constant concentration, C, and depending on the values of the parameters and of C, equation 2 predicts either an exponential bacterial growth followed by saturation or complete bacterial elimination. To account for the regrowth phenomenon observed at certain concentrations, we assumed adaptation of the population with time, through a time-varying EC_{50} (34). This model allowed EC_{50} to vary as a function of both time and ciprofloxacin concentration, according to the following equation:

$$EC_{50} = EC_{50k} \times \alpha \quad (3)$$

with $\alpha = 1 + \beta \times (1 - e^{-C \times \tau \times t})$, where α is the adaptation function, t is time, EC_{50k} is the ciprofloxacin concentration that produces 50% of the maximum rate of death at time zero (before adaptation), β is the maximal adaptation, C is the ciprofloxacin concentration, and τ is the adaptation rate.

Parameter estimation. Parameter estimation was performed using nonlinear mixed-effect regression. Data were log transformed for the analysis. The inter-individual variability of parameters was modeled with an exponential model that constrains parameter estimates to be positive. Residual variability was modeled with a proportional model due to the large range of observed counts and subsequently justified by the inspection of residual plots.

The NONMEM software (NON linear Mixed Effect Model, version V 1.1, Globomax, Hanover, MD) was used for data analysis. The algorithm implemented was the first-order (FO) method with ADVAN6.

Model building. Model building was performed to select the appropriate structure for covariance and fixed effects. First, a base model without variability was fitted, in order to obtain preliminary estimates of all parameters. Then, a diagonal covariance matrix was introduced, so that each parameter was allowed to vary within subjects. This corresponds to the full model. If the full model was overparameterized and successful convergence impossible, then parameters were removed one by one from the model until convergence could be obtained. First variance parameters with estimations either near 0 (<1%) or very large (>200%) were removed one by one. Afterwards, variance parameters and fixed parameters that did not improve significantly the objective function were also removed from the model. Then, if convergence could not be achieved yet or if correlations greater than 95% were found, parameters were removed one by one from the model, starting with parameters with the lowest impact on the objective function (OF). When no correlations were left and convergence was successful, then the structural model was considered as determined. When convergence was achieved, likelihood ratio tests were used to compare nested models during this process. The objective function, OF, is equal up to a constant to minus twice the inverse of the likelihood function, L. Let $(OF_A - OF_B)$ denote the difference between two models, A and B, where B is nested in A. The distribution of $(OF_A - OF_B)$ is approximately a chi-square distribution with a number of degrees of freedom equal to the difference between the number of parameters in the model when testing for a fixed effect and half that of the chi-square distribution for a variance term. The final model was also evaluated using standard goodness-of-fit plots, including individual fits and weighted residuals versus time or predicted concentrations.

RESULTS

The ciprofloxacin MICs of the reference strain of *P. aeruginosa* PAO1 and the PAO7H efflux mutant were estimated at 0.25 μg/ml and 1 μg/ml, respectively.

Twenty-eight kill curves were generated: i.e., 145 bacterial numerations. Fourteen count values were under the limit of quantification (100 CFU/ml). In the absence of ciprofloxacin, the bacterial population reached the maximal number of bacteria (about 2×10^9 CFU/ml) approximately 12 h after the start of the experiment. In the presence of 0.12 μg/ml of ciprofloxacin, the bacterial count grew more slowly than with the controls. At ciprofloxacin concentrations between 0.25 and 1

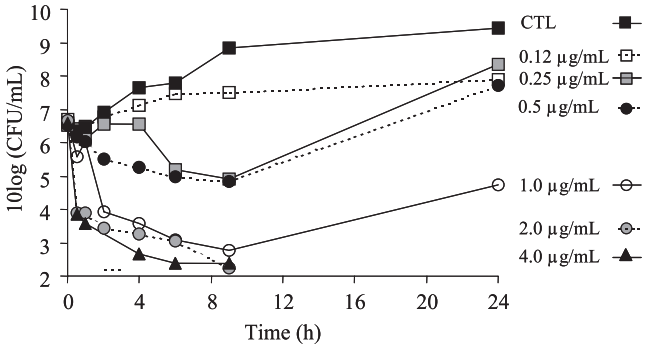

FIG. 1. Representative time-kill curves for *P aeruginosa* exposed to ciprofloxacin at concentrations ranging from 0 to 4.0 μg/ml. CTL, control.

μg/ml, the CFU initially decayed, reached a nadir, and then rebounded, and at ciprofloxacin concentrations ≥2 μg/ml, only a decay of CFU with time was observed. Representative time courses of bacterial burden associated with various ciprofloxacin concentrations are presented in Fig. 1.

Results of bacterial population analysis performed by determining the bacterial survival in the presence of increasing ciprofloxacin concentrations, with or without 24 h of preexposure to the antibiotic, are presented in Table 1. Without preexposure to ciprofloxacin, no bacteria grew with a ciprofloxacin concentration of ≥0.5 μg/ml. Only 0.02% (5×10^5 CFU/ml) of the initial inoculum (1.6×10^8 CFU/ml) grew at the MIC (0.25 μg/ml) and 0.0002% (4×10^2 CFU/ml) grew at 2-fold the MIC (0.5 μg/ml). There was no growth above 2-fold the MIC. After a 24-h preexposure to ciprofloxacin at a concentration of 0.12 μg/ml, 75% of bacteria were able to grow at the MIC and only 1.25% of bacteria grew at 2 μg/ml (8-fold the MIC). Consistent results were observed after 24 h of preexposure to a ciprofloxacin concentration of 0.25 μg/ml, with, respectively, 70% and 0.08% of bacteria able to grow at the ciprofloxacin MIC and at 8-fold the MIC.

The resistance phenotype to ciprofloxacin was obtained by determining the ciprofloxacin susceptibility of bacteria with or without 24 h of preexposure to ciprofloxacin. Without ciprofloxacin preexposure, all bacterial clones showed the same ciprofloxacin susceptibility as the PAO1 wild-type strain (MIC of 0.25 μg/ml) and the same inhibitory effect of the Mex efflux system inhibitor, with the ciprofloxacin MIC being decreased to 0.06 μg/ml in the presence of PAβN (Table 2). After 24 h of preexposure at a ciprofloxacin concentration of 0.12 μg/ml or 0.25 μg/ml, the MIC of the tested clones was increased to 1 to 2 μg/ml. In these strains with low-level resistance, adaptation to ciprofloxacin was mainly due to an overexpression of the Mex efflux pumps, as shown by the 16-fold diminution of the MIC in the presence of PAβN (Table 2). No mutations in the QRDRs of the *gyrA* and *parC* genes were found whatever the time-kill curves, showing the absence of resistance by alteration of the target enzymes.

TABLE 2. Ciprofloxacin susceptibility of bacterial populations with or without 24 h of preexposure to the antibiotic

Bacterial strains	Ciprofloxacin concn (μg/ml):		Ciprofloxacin MIC (μg/ml):	
	Preexposure	During plating expt	Without PAβN	With 40 μg/ml PAβN
PAO1			0.25	0.06
	0	0	0.25	0.06
		0.25	0.25	0.06
	0.12	0	1	0.06
		0.25	1	0.12
		0.5	2	0.12
		1	2	0.12
		2	2	0.12
	0.25	0	1	0.25
		0.25	1	0.12
		0.5	1	0.12
		1	1	0.12
PAO7H			1	0.06

A PK-PD model with adaptation development was used to describe these data. During the model construction, five interindividual variabilities out of seven were removed without significantly increasing the objective function. Interindividual variability was estimated in terms of the parameters k_0 and EC_{50k}. Whatever the ciprofloxacin concentration, the model described adequately the decay and growth phases of CFU counts over time. Diagnostic plots are presented in Fig. 2. Weighted residuals as a function of time were randomly scattered around 0, and adjustments between individual predictions and observed experimental data were globally good, except for a few data points due to the large variability of the experimental data. The precision of the model parameters was satisfactory, but the residual variability was estimated at a very large value of 103%. The final estimates and relative standard errors (RSE) of the model parameters are presented in Table 3.

DISCUSSION

Microbial regrowth after an initial decrease is quite frequently observed with constant antibiotic concentrations, such

TABLE 1. Bacterial counts after 48 h of incubation at 37°C, obtained 24 h after starting the time-kill experiments, in the absence or presence of ciprofloxacin

Ciprofloxacin concn during time-kill expts (μg/ml)	Bacterial count in CFU/ml (% survival) with ciprofloxacin concn of:					
	0 μg/ml	0.25 μg/ml	0.5 μg/ml	1 μg/ml	2 μg/ml	4 μg/ml
0	1.6×10^8	3.2×10^4 (0.02)	4.0×10^2 (0.0002)	0	0	0
0.12	2.0×10^7	1.6×10^7 (75)	7.9×10^6 (40)	1.0×10^6 (5)	2.5×10^5 (1.25)	5.0×10^2 (0.0025)
0.25	4.0×10^5	3.2×10^5 (70)	3.2×10^5 (70)	1.6×10^5 (35)	3.2×10^2 (0.08)	0

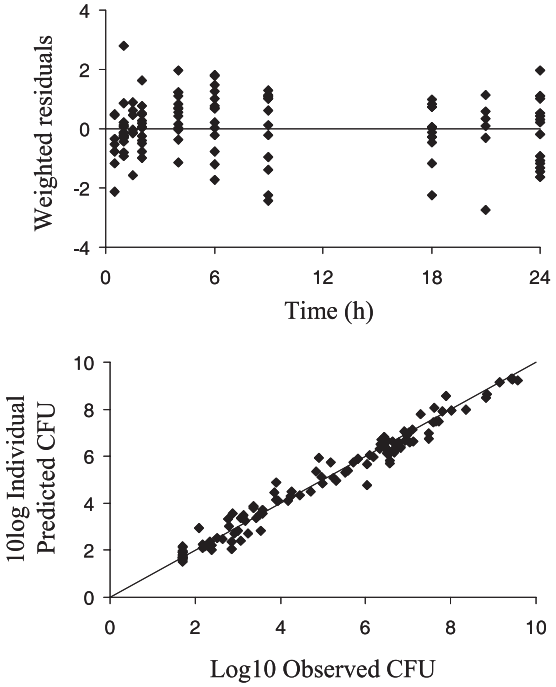

FIG. 2. Weighted residuals versus time and individual predicted CFU versus observed CFU during time-kill studies.

as during time-kill studies, but is rarely addressed in data analysis (4). Such a phenomenon was observed in the present study for ciprofloxacin concentrations between the MIC (0.25 μg/ml) and 8-fold the MIC (2 μg/ml) and cannot be explained by antibiotic degradation since ciprofloxacin is stable several days at 37°C (3). When regrowth occurs, the traditional MIC determination is much less informative than a PK-PD approach, which can capture the dynamic nature of the bacterial susceptibility. Regrowth can be described using either cell proliferation models with cycle-specific inactivation or adaptation models (19). Cell proliferation models consist of separation of the total cell population into proliferating and quiescent subpopulations. They were first used in oncology and were adapted more recently to antimicrobial activity by differentiating susceptible and resistant subpopulations (14). The growth of the resistant subpopulation during exposition to the antimicrobial agent explains the observed regrowth and thus these models may be interpreted as models of development of resistance (7, 15, 25). With adaptation models on the other hand, the susceptibility of the bacteria to the antimicrobial agents is modeled to decrease with time and/or drug concentration. Adaptation was described with various PD parameters such as the growth rate (k_0) (24), the maximum killing rate (k_{max}) (32), or the concentration producing 50% of the maximum effect (EC_{50}) (34). In the present study, we chose the adaptation model in which EC_{50} varies with time and concentration, as previously described by Tam et al. (34), which from a mechanistic point of view seems to be the most appropriate model to reflect a changing bacterial sensitivity to the antibiotic.

To examine whether bacteria have developed known mechanisms of resistance to the antibiotic, we searched for the two mechanisms of FQ resistance frequently found in *P. aeruginosa* that generally exist simultaneously in resistant isolates (13). The first one involves modifications of the DNA gyrase and topoisomerase IV enzymes caused by preferential mutations in the QRDR of *gyrA* and *parC* subunit genes; mutations in the *gyrB* and *parE* subunit genes are rare (2). The second mechanism is a reduced intrabacterial accumulation due to a decreased permeability of the cell wall generally associated with an increased efflux by Mex multidrug resistance (MDR) pumps (30). We used a phenotypic test with the PAβN to explore the last mechanism (16).

Our results showed that 24 h of preexposure to ciprofloxacin at subinhibitory concentrations (0.12 and 0.25 μg/ml) may favor the rapid emergence of bacteria with increased but moderate resistance (an 8-fold increase of ciprofloxacin MIC), simply through acquisition of an overexpression of Mex efflux pumps. This observation is consistent with the fact that this efflux mechanism should be the first mobilized mechanism after 24 h of exposure to the antibiotic, as previously observed by Jumbe et al. (14) and confirmed in a recent study showing that such a type of low-resistance mutant is very prevalent among clinical isolates of *P. aeruginosa* (10). In *P. aeruginosa*, FQ resistance by an active efflux mechanism is mediated by overexpression or expression of multidrug-resistant (MDR) pumps that can confer resistance to a wide spectrum of structurally unrelated compounds. Four MDR systems are responsible for FQ efflux: MexAB OprM, MexCD OprJ, MexEF OprN, and MexXY OprM (33). The PAβN effect in PAO1 is mainly due to the constitutive expression of the MexAB system acting in the intrinsic resistance to FQs, as previously described in genetic inactivation experiments (1, 17). The activity of the MexXY system is known to be unaffected by PAβN (20). Two other Mex systems, MexCD and MexEF, contribute to the acquired resistance to FQs when overexpressed. Molecular analysis of mRNA expression could provide quantitative results to determine the implication level of each Mex system.

Overexpression of Mex efflux pumps, together with the absence of resistance by the alteration of the target enzymes, suggested that a PK-PD model with adaptation was more appropriate to describe these data than a cell proliferation model with two subpopulations (susceptible and resistant). The PK-PD model with adaptation previously proposed by Tam et al. (34) provided a satisfactory fitting of our experimental data. Precision of mean parameter estimates was sat-

TABLE 3. Population pharmacokinetic parameter estimates

Parameter	Parameter		Interindividual variability	
	Estimate	RSE (%)	CV[a] (%)	RSE (%)
k_0 (h^{-1})	0.392	16	39	57
N_{max} (10^9 CFU/ml)	1.93	37		
EC_{50k} (μg/ml)	3.34	26	18	51
Maximal adaptation (β)	56	37		
Rate of adaptation (τ in ml/μg · h)	0.043	41		
k_{max} (h^{-1})	47	24		
γ	1.2	7		
Residual (%)	103	23		

[a] CV, coefficient of variation.

isfactory, with relative standard errors (RSE) relatively low. In contrast, interindividual variability could be estimated only for two parameters (k_0 and EC_{50k}). Considering the large residual variability inherent in the time-kill curve experiments (e.g., 50% variability of the inoculum enumeration), a precise estimation of interindividual variability might necessitate more than the 4 replicates per concentration we performed. The residual value was elevated (103%), but not uncommon for the bacterial count data, as illustrated by the 98% residual value reported by Nielsen et al. (25). These large residual values are deemed to reflect the wide range of bacterial concentrations rather than a poor fit.

According to the model structure, EC_{50} was designed to adapt as a function of time and ciprofloxacin concentration. This adaptation was characterized by its amplitude (α) and rate of adaptation (τ). The α parameter varies with concentration and time, and as an example after exposure of bacteria to a ciprofloxacin concentration equal to 0.25 μg/ml (i.e., the MIC value) for 18 h (time of MIC determination), α was equal to 9, implying a decreased susceptibility or increased EC_{50} from 3.34 to 30.2 μg/ml. For comparisons between studies, the β parameter that reflects maximal adaptation is better suited. The typical value for this parameter was estimated to be 56 in our study, compared with 5.85 obtained by Tam et al. (34) during time-kill studies performed with meropenem and a different strain of *P. aeruginosa*, indicating that the amplitude of adaptation was about 10-fold greater in our study. Regarding the adaptation rate, our typical value for τ was estimated to 0.043 ml/μg · h, compared with 0.0095 ml/μg · h for Tam et al. (34), suggesting that adaptation was more rapid in our experiment. It would now be interesting to determine whether these differences are due the antibiotics or the strains.

However, these results should be interpreted with caution. Although a PK-PD model with adaptation was preferred for reasons previously discussed, cell proliferation models with two subpopulations (susceptible and resistant) could also have been successfully used to analyze our experimental data from a mathematical point of view (data not shown). Furthermore, the probability that a resistant subpopulation exists within a predominantly drug-susceptible wild-type population is dependent on the number of bacteria and the mutational frequency of resistance caused by mechanisms attributable to single point mutations is most frequently between 10^{-6} and 10^{-8}. Consequently a cell proliferation model or a model combining adaptation phenomenon with the presence of two subpopulations (susceptible and resistant) could have been more appropriate with higher initial CFU. On top of that, as a first level of resistance, overexpression of Mex efflux pumps could favor the emergence of mutants with target mutations, often associated with a high resistance level or mutants with a broad-spectrum resistance to other antimicrobial agents (10, 36). It should therefore be important to prevent this early adaptative process, which could be obtained by a relatively modest dosing increase since this loss of sensitivity to ciprofloxacin was rapid but moderate. Complementary experiments over a longer period with concentrations changing with time to mimic the clinical situation, and obtained with the hollow fiber system, should be conducted to confirm this hypothesis and validate the robustness of the semimechanistic PK-PD modeling approach in a larger range of experimental settings, including situations that would mimic clinical conditions/practices.

In conclusion, the MIC is inadequate to assess the susceptibility of a bacterial strain to an antimicrobial agent when adaptation develops quickly, leading to regrowth during time-kill studies. In contrast, PK-PD modeling allows description of this phenomenon and could be used to compare antibiotic efficacies for different bacterial strains and as a tool to optimize the dosing regimen, in particular to prevent the emergence of resistance. However, additional experiments will be required to validate the robustness of this model after longer exposure periods and multiple dosing regimens, as well as *in vivo*.

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